LEAF THICKNESS AND STRUCTURE OF *VITIS VINIFERA* L. CV. ALBARIÑO CLONES AND ITS POSSIBLE RELATION WITH SUSCEPTIBILITY TO DOWNY MILDEW (*PLASMOPARA VITICOLA*) INFECTION

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Abstract

Aims: The grapevine (*Vitis vinifera* L.) cultivar Albariño is currently the most economically important in Galicia (northwestern Spain). Earlier works assessing the natural susceptibility to downy mildew leaf infection (both in the laboratory and in the field), carried out in the collection of Albariño clones at the Misión Biológica de Galicia (CSIC), showed great differences among the clones (Boso *et al*., 2004b, 2005b, 2006; Boso and Kassemeyer, 2008). The aim of the present work is to highlight the histological differences in leaves, in particular thickness and structure, among the 11 different Albariño clones and to find out their possible relation with their natural susceptibility to *Plasmopara viticola.*

Methods and Results: Transverse sections of adult leaves where prepared and observed under light microscope. The area corresponding to the different leaf layers was measured. The results showed significant differences between the clones regarding the thickness of the spongy mesophyll. The clones CSIC-4 and CSIC-1 had the thickest spongy mesophyll (average mean = 14316.8 μm^2) whereas CSIC-3 showed the thinnest one (11548.1 μm^2).

Conclusion: The CSIC-3 clone, one of the least susceptible clones to *P. viticola* in previous studies, showed the thinnest and most compact spongy mesophyll. On the contrary, the CSIC-1 clone had the thickest spongy mesophyll and was also one of the most susceptible to this pathogen. Therefore, it could be possible to relate their histological leaf characteristics with their different levels of natural susceptibility to *P. viticola.*

Significance and impact of the study: This work contributes to the understanding of the link between histological characteristics of leaf layers and mesophyll cells and the different natural susceptibility of grapevines to downy mildew. This may become in the future a valid tool to be used during clonal selections in grapevine breeding programs.

Key words: adult leaves, histology, *Plasmopara viticola*, spongy mesophyll, susceptibility

Résumé

Objectifs: Le cépage Albariño (*Vitis vinifera* L.) est actuellement le plus important économiquement en Galice (Nord-Ouest de l'Espagne). Des études préalables sur la sensibilité naturelle au mildiou des feuilles (au laboratoire et au champ) d'une collection de clones d'Albariño de la Misión Biológica de Galicia (CSIC) a montré des différences entre clones (Boso *et al*., 2004b, 2005b, 2006; Boso et Kassemeyer, 2008). Le but de cette étude est d'évaluer si les différences de sensibilité au mildiou observées entre clones de l´Albariño peuvent être reliées à des différences au niveau histologique, en particulier de l'épaisseur et de la structure de la feuille.

Méthodes et résultats: Des sections transversales de feuilles adultes ont été préparées et observées au microscope optique. L'aire de chacun des tissus de la feuille a été mesurée. Les résultats ont montré des différences significatives entre les clones pour l'épaisseur du parenchyme lacuneux. Les clones CSIC-4 et CSIC-1 présentaient les parenchymes les plus épais (moyenne = 14316.8 μm^2) et le clone CSIC-3 le parenchyme plus fin (11548.1 μm^2).

Conclusion: Le clone CSIC-3, le moins susceptible au mildiou dans les études antérieures, a montré le parenchyme lacuneux le plus fin et le plus compact. Au contraire, CSIC-1 dont le parenchyme s'est révélé le plus épais était l'un des clones les plus sensibles au mildiou. Ces résultats suggèrent l'existence d'une possible relation entre les caractéristiques histologiques des clones d'Albariño et les différents niveaux de sensibilité naturelle au mildiou.

Signification et impact de l'étude: Ce travail éclaire la relation entre les caractéristiques histologiques de différents tissus de la feuille et le niveau de susceptibilité des feuilles de vigne au mildiou. Dans le futur, ces résultats pourraient s'avérer utile pour les programmes de sélection clonale.

Mots clés: feuilles adultes, histologie, *Plasmopara viticola*, parenchyme lacuneux, susceptibilité

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INTRODUCTION

The Albariño (*Vitis vinifera* L.) grapevine cultivar is currently the most economically important in Galicia (northwestern Spain), where it is very well adapted to the soil and climatic conditions. Galicia has abundant rainfall and mild temperatures and attacks by *Plasmopara viticola* (downy mildew) are very frequent. Therefore, fungicides have to be applied several times during the growing season, greatly increasing production costs and reducing growers' profits.

Since 1987, the Misión Biológica de Galicia (CSIC) has been involved in studies on the intravarietal variation shown by Albariño (Martínez *et al*., 2005) in terms of its ampelographic, agronomic and molecular properties (Loureiro, 1999; Boso *et al*., 2004a, 2005a, 2007) and its susceptibility to natural cryptogamic diseases, particularly to downy mildew (Boso *et al*., 2004b, 2005b, 2006; Boso and Kassemeyer, 2008).

At the macroscopic level, numerous studies reported great differences among the varieties of *V. vinifera* L. (Foëx, 1891; Huglin, 1986; Galet, 2000), as well as between different *Vitis* species (*V. vinifera*, *V. riparia*, etc.). However, comparative studies on grapevine histology, especially on the characteristics of leaf tissues and mesophyll cells of different Vitis species, are still lacking. In the literature, there are only a few publications on cv. Carignan (Bernard, 1978), on other French varieties (Galet, 2000) and on Eastern European cultivars (Codreanu, 2006). None was found on cv. Albariño in this aspect. The work of Bernard (1978) is perhaps the most important in this area, providing descriptions of the histological characteristics of the leaves of *V. vinifera* L. cv. Carignan and how they change during the growth cycle. Codreanu (2006) compared the characteristics of leaf tissues belonging to different species of Vitis, as well as those of a number of Eastern European varieties of *V. vinifera*. Palliotti *et al*. (2000) studied the histological differences between green and lateral shoot leaves, and finally, Ben Salem-Fnayou *et al*. (2005) compared the tissues of leaves belonging to the same variety but cultivated in areas with different climate. However, as far as we know, no reports have been published about the leaf tissues of cv. Albariño or even between clones of the same species.

Plasmopara viticola, the causal agent of grapevine downy mildew, is one of the major diseases affecting European viticulture. Different authors (Ravaz, 1914; Boubals, 1959; Ribéreau-Gayon and Peynaud, 1971; Galet, 1995; Staudt and Kassemeyer, 1995; Wiedemann-Merdinoglu *et al*., 2006; Cadle-Davidson, 2008) have shown that there are great differences within the Vitis genus in terms of susceptibility to this disease. While most varieties of *V. vinifera* L. are highly susceptible to downy mildew, other species such as *V. aestivalis, V. arizonica, V. berlandieri, V. doniana, V. palmata* and *V. rupestris* are resistant and some, such as *V. candicans, V. cinerea*, *V. cordifolia, V. monticola, V. riparia, V. rotundifolia* and *V. titania* are highly resistant. From the middle of the 20th century, many studies tried to determine if there were differences among the different *V. vinifera* L. varieties in terms of their level of resistance or susceptibility to downy mildew (Boubals, 1959; Galet, 1977; Li, 1993; Staudt and Kassemeyer, 1995; Staudt, 1997). Although these studies were of great use, they were limited to the most internationally known varieties and used subjective visual parameters largely based on the results of field studies.

According to some authors (Ribéreau-Gayon and Peynaud, 1982; Kortekamp and Zyprian, 2003), the development of the mycelium of downy mildew spreading inside the plant may depend on the degree of the host susceptibility and the tissue type in which the infection takes place (faster in young tissues and slower in lignified ones). The aim of the present work was to determine whether the 11 Albariño clones of our collection display any clear and objective differences at the leaf histology level and if these differences are related to the different degree of susceptibility to downy mildew recorded for these same clones in previous studies.

MATERIALS AND METHODS

1. Plant material

The plant material assessed in this work was 11 clones of cv. Albariño known as CSIC-1, CSIC-2, CSIC-3, CSIC-4, CSIC-5, CSIC-6, CSIC-7, CSIC-8, CSIC-9, CSIC-10 and CSIC-11. All are maintained in the experimental plot at the Misión Biológica de Galicia (CSIC) in Pontevedra, in the Spanish region of Galicia (42° 25´ N, 8° 38´ W; mean annual temperature 14.2°C and mean annual rainfall 1650 mm, with strong annual variation). All of them come from centuries-old plants found around the region (Martínez *et al*., 2005). In April of 1994, these clones were grafted (10 replicates per clone). All are grown en espalier and under Sylvoz pruning system. All receive the same plant protection treatments and are under identical cultivation practices.

In 2005 and 2007, five plants per clone were selected and from each, the 8th leaf from the base of a fertile green shoot was sampled. All leaves were collected between budburst and veraison (July).

2. Microscopy techniques

a) Light microscopy

In each year of study, a 0.3 x 1 cm sample was taken from each of the five leaves per clone. These samples

were taken from the leaf blade close to the petiolar sinus, between the main vein and the first right lateral vein (Figure 1). All samples were immediately fixed in FAE (formalin-acetic acid-ethanol) (Jensen, 1962). All samples were then dehydrated in a standard tertiary butyl alcohol series (50-70-85-95-100%, 12 h per concentration) (Jensen, 1962; D'Ambrogio de Argüeso, 1986), embedded in Paraplast Plus embedding paraffin (melting point 56 °C, Kendall, Mansfield, U.S.A.), cut into 10 μm thick transverse sections and stained with toluidine blue (0.05% v/v aqueous, No. 52040 Panreac Química, SA, Barcelona, Spain) for 15 min prior to paraffin removal (Sakai, 1973). All sections were observed using a Nikon Eclipse E200 light microscope and pictures were taken and analysed using NIS-Elements Basic Research v 2.34 Software (Nikon Instruments Inc., Melville, U.S.A.).

On each picture, a rectangle measuring 120 μm of width by variable height (depending on the thickness of the leaf) was drawn. Within this rectangle, the areas corresponding to the cuticle + adaxial epidermis, the palisade mesophyll, the spongy mesophyll and the abaxial epidermis + cuticle were measured using the NIS-Elements Basic Research v 2.34 Software (Nikon Instruments Inc., Melville, U.S.A.). These measurements provided the average thickness of the different leaf layers.

b) Electron microscopy

To study the cellular organization of the leaf tissues, more samples were obtained as above but fixed in 3% glutaraldehyde in 0.025 M cacodylate buffer (pH 7.5) for 2 h at room temperature and post-fixed in 1% OsO4 in the same buffer for 2 h. After fixation, all samples were washed several times in cacodylate buffer, dehydrated in an ethanol series (as for light microscopy) and embedded in Epon resin. Ultrathin sections (1 μm) were cut using a Reichert-Jung Ultracut E microtome and stained for general contrast with 2% uranyl acetate followed by lead citrate. Observations were made using a Jeol TEM-1011 transmission electron microscope.

3. Downy mildew susceptibility evaluation

This parameter was evaluated in previous studies. Boso *et al*. (2004b, 2005b) first evaluated the natural susceptibility of the 11 Albariño clones to *P. viticola* in the field and then in the laboratory and greenhouse after artificial inoculations (Boso *et al*., 2006). Briefly, the different techniques that had been used for this purpose were as follows:

a) Leaf sampling in the field

Natural infections were used since the disease appears very often in the study location. Fifty adult leaves with symptoms of downy mildew infection were randomly selected from the basal, medial and apical parts of the vine shoots of the 10 plants of each clone (five leaves per plant). Pictures of the complete surface of each leaf were taken using a digital camera. In each picture the number of « oil » spots per leaf was recorded and the leaf area occupied by each spot was measured, as well as the total leaf surface area, using the analySIS 3.0 software (Soft Imaging System GmbH, 1998). From these data, the severity index of leaf infection ([sum of the surface area of all spots/leaf surface area] x 100) was calculated.

b) Leaf disc test in the laboratory

Leaf discs were prepared according to Staudt and Kassemeyer (1995) and Rumbolz *et al*. (2002). The leaf surface was sterilised in 75% ethanol and subsequently washed three times in distilled water. After drying with filter paper, 12 discs (16 mm diameter) were excised per leaf using a cork borer. Discs were placed bottom side up in Petri dishes containing water agar (0.8%). Each disc was inoculated with 50 μl of the sporangia suspension (40,000 sporangia·ml^{-1} in distilled water) and incubated for 5 days in a culture chamber at 25 ºC, photoperiod: 16 h light (2.5 W·m^{-2}). All preparations were repeated in triplicate. Infection symptoms after 5 days, such as sporulation, were scored following the same system as for severity index in leaf test in the field.

c) Detached leaf test in the laboratory

The same procedure as in the leaf disc test was used except that whole leaves were placed on the 0.8% agar (Kiefer *et al*., 2002). Leaves were inoculated with 50 μl drops of the sporangia suspension and incubated in the same conditions as described before in a culture chamber. All preparations were repeated in triplicate. Observations were made after 5 days of incubation and the infection symptoms were scored as described above.

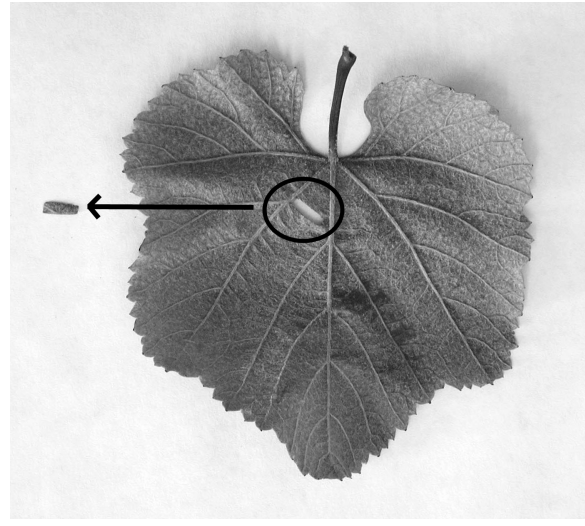

Figure 1. Lower leaf surface: sample for histological study.

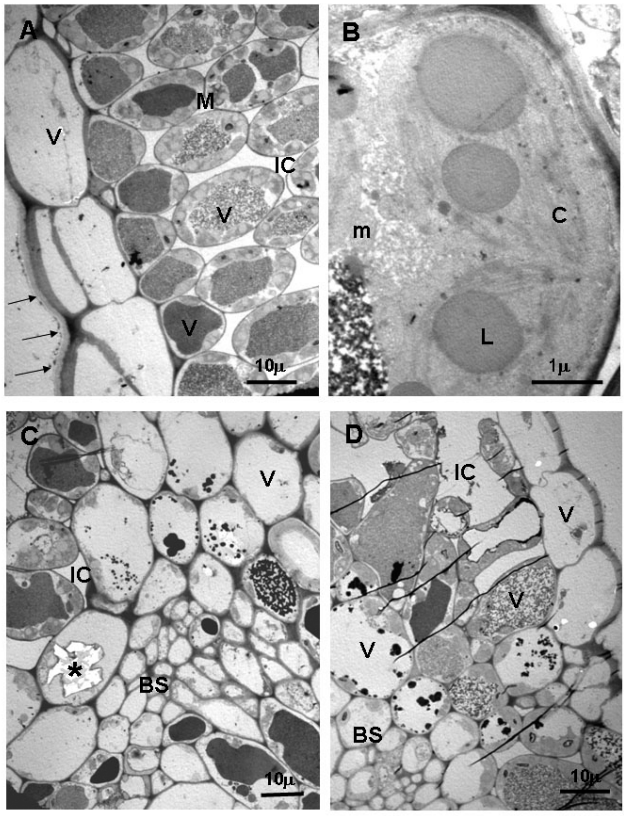

Figure 2. Transmission electron micrographs illustrating Albariño leaf tissues.

A: Adaxial epidermis and palisade mesophyll, B: Chloroplasts adjacent to the cell wall, C: Spongy mesophyll, D: Abaxial epidermis and spongy mesophyll.

BS, bundle sheath; C, chloroplast; IC, intracellular spaces; L, lipid body; M, mesophyll cells; m, mitochondrion; V, vacuole; *, crystal inclusions; ➛, external slight thick cuticle.

d) Plant test in the greenhouse

All cv. Albariño clones were planted in pots in the greenhouse (one plant of each clone) and control plants were sprayed with a suspension of sporangia (40,000 sporangia·ml^{-1} in distilled water). The incubation and evaluation of disease symptoms (apparition of « oil » spots and sporulation) were carried out as described above after 5 days of incubation. All experiments were repeated in duplicate.

4. Statistical analysis

All variables were submitted to analysis of variance (ANOVA) using the data of both years together (2005+2007). When the interaction clone x year was significant, the data for each year of study were analysed separately. The sources of variation were 'clone' (fixed factor) and 'year' (random factor). Fisher's protected test (least significant difference method [LSD]) was used to determine which clones had the thickest and thinner areas of each tissue. Finally, simple correlation analysis among mesophyll layers and disease traits was made in order to determine which trait better explains the mesophyll thickness in the Albariño clones studied. All calculations were undertaken using the SAS System v 9.1 Software (SAS Institute Inc., Cary, U.S.A.).

RESULTS

Under light microscope, leaf sections generally showed a one-cell-thick adaxial epidermis with elongated and thin-walled cells positioned horizontally. The cuticle was slightly thicker compared to the middle lamella of the epidermal cells (Figure 2A). However, the internal organization of these cells may differ among the clones (Figure 3). The palisade mesophyll consisted of elongated cells with the peripheral cytoplasm largely occupied by chloroplasts and sometimes showing many lipid bodies or plastoglobuli (Figure 2B) and occasionally starch granules. The content of the vacuoles was also variable in terms of the quantity of proteinaceous electron-dense inclusions, tannins and other materials of unknown composition (Figure 2A). Lying below the palisade mesophyll was the spongy mesophyll, made up of 4-5 layers of loosely arranged cells of irregular shape, vacuolated and with intracellular spaces between them (Figure 2C). The size of these spaces, and therefore the compactness of the spongy mesophyll, differed between the clones (Figure 3). These cells contained fewer chloroplasts than those of the palisade layer. Crystal inclusions were sometimes seen in the vacuoles of cells near the bundle sheath (Figure 2C), probably of calcium oxalate nature. Cells horizontally arranged were commonly seen between the spongy mesophyll and the abaxial epidermis. The cells of the abaxial epidermis were smaller, similar to those of the adaxial surface, but with bigger intracellular spaces and covered by a cuticle somewhat thinner than that covering the upper epidermis (Figure 2D).

From the statistical analyses, the interaction clone x year was found to have no significant effect on any of the variables examined. Thus, only the results of the overall ANOVA (both years of study analysed together) were considered. The only significant differences ($P < 0.05$) observed between clones were for the thickness of the spongy mesophyll (Table 1). This tissue was significantly thicker in the CSIC-4 and CSIC-1 clones (average mean = 14316.8 μm^2) than in the other clones. Particularly, the CSIC-3 clone had the thinnest (11548.1 μm^2) and most compact spongy mesophyll (Table 2).

As expected, differences were seen between both years of the experiment. The areas corresponding to the different tissues were significantly larger in 2005 than in 2007 (Table 3). However, the differences between clones were

maintained: those with the thickest and thinnest spongy mesophyll were the same in both years (data not shown).

Regarding to the natural susceptibility to *P. viticola* evaluated in previous studies of Boso *et al.* (2004b, 2006), significant differences were observed for the severity index to downy mildew between the clones after natural infection and artificial inoculation in leaves (Table 4). The CSIC-3 and CSIC-6 clones were found to be the least susceptible to downy mildew infection, whereas the CSIC-1, CSIC-2, CSIC-8, CSIC-9 and CSIC-11 clones were the most susceptible. The others showed intermediate susceptibility to the pathogen.

Nevertheless, significant correlations between the thickness of the mesophyll layers and the different

Table 1. Least significant differences (LSD) between the 11 clones resulting from ANOVA for the area of the different leaf tissues.

Source of variation	Variables							
	Cuticle-adaxial epidermis		Palisade mesophyll		Spongy mesophyll		Cuticle-abaxial epidermis	
	df	MS	df	MS	df	MS	df	MS
Clone	10	93519 NS	10	3888447 NS	10	8549334 *	10	78593 NS
Year	1	1623809 ***	1	12461849 *	1	33619798 **	1	6271227 ***
Clone × year	10	66647 NS	10	2016597 NS	10	2296422 NS	10	90436 NS
Error	86	112105	86	1551466	86	3618363	86	71902

Cuticle-adaxial epidermis: area occupied by the cuticle+adaxial epidermis; Palisade mesophyll: area occupied by the palisade mesophyll; Spongy mesophyll: area occupied by the spongy mesophyll; Cuticle-abaxial epidermis: area occupied by the cuticle+abaxial epidermis.
df: degrees of freedom; MS: mean squares; Clone x year: interaction clone x year; *, **, ***: significant at P<0.05, 0.01 and 0.001, respectively; NS: not significant.

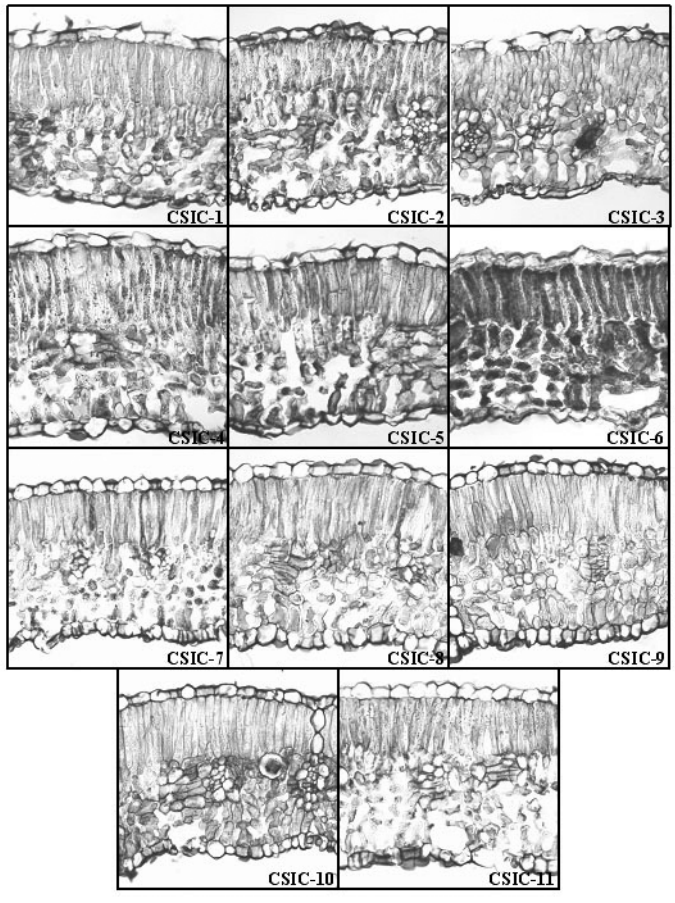

Figure 3. Paraffin leaf sections of the 11 Albariño clones stained with toluidine blue.

susceptibility to *P. viticola* could not be established in these clones.

DISCUSSION

Our work on this subject, which began 20 years ago, has shown the presence of a wide variation within clones of centenarian cv. Albariño plants (Martínez *et al*., 2005). Previous studies led to the selection of 11 clones with different features and their conservation in the collection of the Misión Biológica de Galicia (CSIC). Therefore these clones were planted in the same plot and once they were in full production, new studies were undertaken to confirm their ampelographic (Martínez *et al*., 2005) and agronomic (Boso *et al*., 2004a, 2005a, 2007) differences. In addition, studies to evaluate the susceptibility of this cultivar to *P. viticola*, and particularly of these 11 clones, were made (Boso *et al*., 2004b, 2005b, 2006). Compared to other European cultivars such as Pinot Noir, Pinot Blanc, Cabernet-Sauvignon, Riesling, etc., cv. Albariño was found to be among them the most susceptible (Boso

Table 2. Mean values and least significant differences (LSD) for the area of the spongy mesophyll in the different clones.

Clone	Area of the spongy mesophyll (μm^2)
CSIC-1	14055.9 AB*
CSIC-2	12359.7 BC
CSIC-3	11548.1 C
CSIC-4	14577.6 A
CSIC-5	13196.8 ABC
CSIC-6	12022.9 C
CSIC-7	12230.1 C
CSIC-8	12273.7 C
CSIC-9	12013.0 C
CSIC-10	11915.8 C
CSIC-11	12188.4 C
LSD (0.05)	1708.1

*Values followed by the same letter are not significantly different.

Table 3. Mean values and least significant differences (LSD) for the area of each tissue in each sampling year.

Year	Area (μm^2)			
	Cuticle-adaxial epidermis	Palisade mesophyll	Spongy mesophyll	Cuticle-abaxial epidermis
2005	1995.86 A*	7621.80 A	13140.90 A	1916.42 A
2007	1749.88 B	6918.70 B	11996.20 B	1431.99 B
LSD (0.05)	128.09	476.53	727.74	102.59

*Values followed by the same letter are not significantly different.

Table 4. Severity index of 11 Albariño clones after natural infection and artificial inoculation in leaves with downy mildew (Boso *et al*., 2004b, 2006).

	Artificial inoculation				Natural infection
Clone	Leaf disc	Leaf	Plant[a]	Mean ± Deviation	Plant[b]
CSIC-1	80.00	70.00	36.43	62.14 ± 22.82	43
CSIC-2	100.00	96.67	29.13	75.27 ± 39.99	44
CSIC-3	53.30	53.33	37.05	47.79 ± 9.30	25
CSIC-4	76.67	75.00	25.74	59.14 ± 28.93	43
CSIC-5	76.67	76.67	32.01	61.78 ± 25.78	45
CSIC-6	51.67	50.00	33.63	45.10 ± 9.97	27
CSIC-7	76.67	75.00	34.28	61.98 ± 24.01	48
CSIC-8	76.67	75.00	24.85	58.84 ± 29.45	40
CSIC-9	86.67	78.33	37.10	67.37 ± 26.54	38
CSIC-10	76.67	75.00	32.94	61.54 ± 24.78	31
CSIC-11	76.67	63.33	29.97	56.66 ± 24.05	44

aPotted plants in greenhouse from cuttings; bField plants

and Kassemeyer, 2008). In addition, it was found that some clones of cv. Albariño were more sensitive than others. This was confirmed in the field (Boso *et al*., 2004b, 2005b) and later in the laboratory (Boso *et al*., 2006). Although some authors reported differences between different vine species (Denzer *et al*., 1995a, b; Staudt and Kassemeyer, 1995; Spring *et al*., 1998) and even between different varieties of *V. vinifera* L. (Gindro *et al*., 2003, 2006), differences between clones of the same cultivar have not been reported before.

Different ideas have been proposed to explain the differences between vine species in terms of their susceptibility to fungal diseases. In the second half of the 20th century, the reason for this behaviour attempted to be in anatomic or histological features visible under light microscope (Nysterakis, 1943, cited in Ribéreau-Gayon and Peynaud, 1982; Manzoni, 1954; Bernard, 1978). Progress in the development of electron microscope and plant sample processing allowed the in-depth study of these differences (Chambers and Possingham, 1963; Kortekamp *et al*., 1998; Musetti *et al*., 2005; Codreanu, 2006; Gindro *et al*., 2006; Alonso-Villaverde *et al*., 2011a). However, with the rise of biochemical and molecular techniques in the 1970s and later the arrival of genomic and proteomic tools (Boubals, 1959; Wiedemann-Merdinoglu *et al*., 2006), such kind of studies were abandoned before they could provide reliable conclusions.

As *P. viticola* reached Europe from America in 1878 (Johnson, 1989), it has coexisted with European grapevines around 130 years, which is very little time for plants to develop defence mechanisms. Meanwhile, American vines have coexisted with the pathogen for million years, which enabled them to develop a certain level of adaptation with different levels of resistance to the pathogen according to the species (Boubals, 1959; Galet, 1995). Therefore, the differences observed between different grapevine cultivars, and between the different clones of Albariño, could be due to the intrinsic characteristics of these plants rather than any adaptation to the presence of the pathogen. Different varieties and clones may have intrinsic barriers that the pathogen finds more or less difficult to overcome. These barriers might be found in the internode distances of green shoots (Palliotti *et al*., 2000), the presence or absence of certain types of hairs on the leaves (Kortekamp and Zyprian, 1999; Kortekamp, 2003), the number of stomata (Allègre *et al*., 2006; Alonso-Villaverde *et al*., 2011b), the characteristics of the different tissues making up the leaf mesophyll (regarding to downy mildew infection of the leaf) or the density of the fruit clusters or the size of the cluster pedicels (regarding to cluster infection). Considering leaf hairiness, studies performed by our group (Martínez *et al*., 2005) have shown that the 11 clones evaluated in the present work have the same density of reclining and erect hairs on both sides. Moreover, fluorescence microscopy observations made by Boso *et al*. (2006) suggest that no relationship exists between hair density and susceptibility to downy mildew in these 11 clones or other varieties.

The present results show that cv. Albariño have a leaf mesophyll structure that corresponds to that described by Bernard (1978) and Galet (2000) for a number of *V. vinifera* L. varieties.

Some authors (Nysterakis, 1943, cited in Ribéreau-Gayon and Peynaud, 1982) suggest that the leaves of different varieties of *V. vinifera* might differ in thickness and that the anatomical structure of the spongy mesophyll might influence the growth of *P. viticola* inside the leaf. Growth might be limited if the cells of the tissue are very compact, leaving very few and little intercellular spaces in which haustoria could develop. Accordingly, the CSIC-1 clone, which is one of the most susceptible to downy mildew (Boso *et al*., 2004b, 2006), showed the thickest spongy mesophyll along with the CSIC-4 clone. On the other hand, the CSIC-3 clone is one of the least susceptible to downy mildew and showed the thinnest and most compact spongy mesophyll. Therefore, it seems that there is an effect of the anatomy of the mesophyll of the leaves on the susceptibility to downy mildew infection.

Although other authors have observed differences between the thickness of the epidermis, hypodermis and cuticle and indicated the existence of a positive correlation between resistance to cryptogamic diseases and the thickness of these layers (Gabler *et al*., 2003), in the present work, no significant statistical correlations between the thickness of the different leaf layers and the different susceptibility to *P. viticola* for these clones could be established. This is due to the difficulty to establish different degrees of susceptibility to *P. viticola* within cultivars of susceptible pattern.

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